PCT/US00/12061

ludet . Ludbul

## **CLAIMS**

What is claimed is:

1. An isolated polynucleotide comprising a nucleotide sequence selected from the group consisting of:

- (a) a first nucleotide sequence encoding a polypeptide of at least 30 amino acids that has at least 85% identity based on the Clustal method of alignment when compared to a polypeptide of SEQ ID NO:6;
- (b) a second nucleotide sequence encoding a polypeptide of at least 50 amino acids that has at least 80% identity based on the Clustal method of alignment when compared to a polypeptide selected from the group consisting of SEQ ID NOs:16, 28, 36, and 40;
- (c) a third nucleotide sequence encoding a polypeptide of at least 50 amino acids that has at least 85% identity based on the Clustal method of alignment when compared to a polypeptide of SEQ ID NO:12;
- (d) a fourth nucleotide sequence encoding a polypeptide of at least 50 amino acids that has at least 90% identity based on the Clustal method of alignment when compared to a polypeptide selected from the group consisting of SEQ ID NOs:8 and 24;
- (e) a fifth nucleotide sequence encoding a polypeptide of at least 50 amino acids that has at least 95% identity based on the Clustal method of alignment when compared to a polypeptide selected from the group consisting of SEQ ID NOs:18 and 32;
- (f) a sixth nucleotide sequence encoding a polypeptide of at least 90 amino acids that has at least 95% identity based on the Clustal method of alignment when compared to a polypeptide of SEQ ID NO:42;
- (g) a seventh nucleotide sequence encoding a polypeptide of at least 95 amino acids that has at least 95% identity based on the Clustal method of alignment when compared to a polypeptide of SEQ ID NO:46;
- (h) an eighth nucleotide sequence encoding a polypeptide of at least 100 amino acids that has at least 80% identity based on the Clustal method of alignment when compared to a polypeptide selected from the group consisting of SEQ ID NO:20;
- (i) a ninth nucleotide sequence encoding a polypeptide of at least 100 amino acids that has at least 90% identity based on the Clustal method of alignment when compared to a polypeptide of SEQ ID NO:2;
- (j) a tenth nucleotide sequence encoding a polypeptide of at least 150 amino acids that has at least 95% identity based on the Clustal method of alignment when compared to a polypeptide of SEQ ID NO:4;

10

5

20

25

30

35

5

10

15

20

25

30

PCT/US00/12061

(k) an eleventh nucleotide sequence encoding a polypeptide of at least 300 amino acids that has at least 80% identity based on the Clustal method of alignment when compared to a polypeptide of SEQ ID NO:38;

- (l) a twelfth nucleotide sequence encoding a polypeptide of at least 350 amino acids that has at least 95% identity based on the Clustal method of alignment when compared to a polypeptide of SEQ ID NO:10;
- (m) a thirteenth nucleotide sequence encoding a polypeptide of at least 400 amino acids that has at least 80% identity based on the Clustal method of alignment when compared to a polypeptide selected from the group consisting of SEQ ID NOs:22, 26 and 30;
- (n) a fourteenth nucleotide sequence encoding a polypeptide of at least 500 amino acids that has at least 80% identity based on the Clustal method of alignment when compared to a polypeptide of SEQ ID NO:34;
- (o) a fifteenth nucleotide sequence encoding a polypeptide of at least 200 amino acids having at least 80% identity based on the Clustal method of alignment when compared to a polypeptide of SEQ ID NO:14;
- (p) a sixteenth nucleotide sequence encoding a polypeptide of at least 250 amino acids having at least 90% identity based on the Clustal method of alignment when compared to a polypeptide of SEQ ID NO:48; and
- (q) a seventeenth nucleotide sequence comprising a complement of (a), (b), (c), (d), (e), (f), (g), (h), (i), (j), (k), (l), (m), (n), (o) or (p).
- 2. The isolated polynucleotide of Claim 1, wherein the first nucleotide sequence comprises a nucleic acid sequence selected from the group consisting of SEQ ID NOs:1, 3, 5, 7, 9, 11, 13, 15, 17, 19, 21, 23, 25, 27, 29, 31, 33, 35, 37, 39, 41, 45, and 47 that codes for the polypeptide selected from the group consisting of SEQ ID NOs:2, 4, 6, 8, 10, 12, 14, 16, 18, 20, 22, 24, 26, 28, 30, 32, 34, 36, 38, 40, 42, 46, and 48.
- 3. The isolated polynucleotide of Claim 1 wherein the nucleotide sequences are DNA.
- 4. The isolated polynucleotide of Claim 1 wherein the nucleotide sequences are RNA.
  - 5. A chimeric gene comprising the isolated polynucleotide of Claim 1 operably linked to at least one suitable regulatory sequence.
    - 6. A host cell comprising the chimeric gene of Claim 5.
    - 7. A host cell comprising the isolated polynucleotide of Claim 1.
- 8. The host cell of Claim 7 wherein the host cell is selected from the group consisting of yeast, bacteria, and plant.
  - 9. A virus comprising the isolated polynucleotide of Claim 1.
  - 10. A polypeptide selected from the group consisting of:

35

PCT/US00/12061

on the Clustal method of alignment when compared to a polypeptide of SEO ID NO:6; (b) a polypeptide of at least 50 amino acids that has at least 80% identity based on the Clustal method of alignment when compared to a polypeptide 5 selected from the group consisting of SEQ ID NOs:16, 28, 36, and 40; (c) a polypeptide of at least 50 amino acids that has at least 85% identity based on the Clustal method of alignment when compared to a polypeptide of SEO ID NO:12; (d) a polypeptide of at least 50 amino acids that has at least 90% identity based 10 on the Clustal method of alignment when compared to a polypeptide selected from the group consisting of SEO ID NOs:8 and 24: (e) a polypeptide of at least 50 amino acids that has at least 95% identity based on the Clustal method of alignment when compared to a polypeptide 15 selected from the group consisting of SEQ ID NOs:18 and 32; (f) a polypeptide of at least 90 amino acids that has at least 95% identity based on the Clustal method of alignment when compared to a polypeptide of SEO ID NO:42; (g) a polypeptide of at least 95 amino acids having at least 95% identity based 20 on the Clustal method of alignment when compared to a polypeptide of SEO ID NO:46: (h) a polypeptide of at least 100 amino acids that has at least 80% identity based on the Clustal method of alignment when compared to a polypeptide selected from the group consisting of SEQ ID NO:20; (i) a polypeptide of at least 100 amino acids that has at least 90% identity based 25 on the Clustal method of alignment when compared to a polypeptide of SEO ID NO:2; (j) a polypeptide of at least 150 amino acids that has at least 95% identity based on the Clustal method of alignment when compared to a polypeptide of SEQ 30 ID NO:4; (k) a polypeptide of at least 300 amino acids that has at least 80% identity based on the Clustal method of alignment when compared to a polypeptide of SEO ID NO:38; (l) a polypeptide of at least 350 amino acids that has at least 95% identity based

(a) a polypeptide of at least 30 amino acids that has at least 85% identity based

ID NO:10;

on the Clustal method of alignment when compared to a polypeptide of SEO

WO 00/68389 PCT/US00/12061

5

10

15

20

30

35

(m) a polypeptide of at least 400 amino acids that has at least 80% identity based on the Clustal method of alignment when compared to a polypeptide selected from the group consisting of SEQ ID NOs:22, 26 and 30;

- (n) a polypeptide of at least 500 amino acids that has at least 80% identity based on the Clustal method of alignment when compared to a polypeptide of SEQ ID NO:34;
- (o) a polypeptide of at least 200 amino acids having at least 80% identity based on the Clustal method of alignment when compared to a polypeptide of SEQ ID NO:14; and
- (p) a polypeptide of at least 250 amino acids having at least 90% identity based on the Clustal method of alignment when compared to a polypeptide of SEQ ID NO:48.
- 11. A method of selecting an isolated polynucleotide that affects the level of expression of a polypeptide in a plant cell, the method comprising the steps of:
  - (a) constructing the isolated polynucleotide comprising a nucleotide sequence of at least one of 30 contiguous nucleotides derived from the isolated polynucleotide of Claim 1;
  - (b) introducing the isolated polynucleotide into the plant cell;
  - (c) measuring the level of the polypeptide in the plant cell containing the polynucleotide; and
  - (d) comparing the level of the polypeptide in the plant cell containing the isolated polynucleotide with the level of the polypeptide in a plant cell that does not contain the isolated polynucleotide.
- 12. The method of Claim 11 wherein the isolated polynucleotide consists of the nucleotide sequence selected from the group consisting of SEQ ID NOs:1, 3, 5, 7, 9, 11, 13, 15, 17, 19, 21, 23, 25, 27, 29, 31, 33, 35, 37, 39, 41, 45, and 47 that codes for the polypeptide selected from the group consisting of SEQ ID NOs:2, 4, 6, 8, 10, 12, 14, 16, 18, 20, 22, 24, 26, 28, 30, 32, 34, 36, 38, 40, 42, 46, and 48.
  - 13. A method of selecting an isolated polynucleotide that affects the level of expression of a polypeptide in a plant cell, the method comprising the steps of:
    - (a) constructing the isolated polynucleotide of Claim 1;
    - (b) introducing the isolated polynucleotide into the plant cell;
    - (c) measuring the level of the polypeptide in the plant cell containing the polynucleotide; and
    - (d) comparing the level of the polypeptide in the plant cell containing the isolated polynucleotide with the level of the polypeptide in a plant cell that does not contain the polynucleotide.

WO 00/68389 PCT/US00/12061

14. A method of obtaining a nucleic acid fragment encoding a polypeptide comprising the steps of:

- (a) synthesizing an oligonucleotide primer comprising a nucleotide sequence of at least one of 30 contiguous nucleotides derived from a nucleotide sequence selected from the group consisting of SEQ ID NOs:1, 3, 5, 7, 9, 11, 13, 15, 17, 19, 21, 23, 25, 27, 29, 31, 33, 35, 37, 39, 41, 45, and 47 and a complement of such nucleotide sequences; and
- (b) amplifying the nucleic acid sequence using the oligonucleotide primer.
- 15. A method of obtaining a nucleic acid fragment encoding a polypeptide comprising the steps of:
  - (a) probing a cDNA or genomic library with an isolated polynucleotide comprising at least one of 30 contiguous nucleotides derived from a nucleotide sequence selected from the group consisting of SEQ ID NOs:1, 3, 5, 7, 9, 11, 13, 15, 17, 19, 21, 23, 25, 27, 29, 31, 33, 35, 37, 39, 41, 45, and 47 and a complement of such nucleotide sequences;
  - (b) identifying a DNA clone that hybridizes with the isolated polynucleotide;
  - (c) isolating the identified DNA clone; and

5

15

20

25

30

- (d) sequencing a cDNA or genomic fragment that comprises the isolated DNA clone.
- 16. A method for evaluating at least one compound for its ability to inhibit the activity of a protein, the method comprising the steps of:
  - (a) transforming a host cell with a chimeric gene comprising a nucleic acid fragment encoding the polypeptide, operably linked to at least one suitable regulatory sequence;
  - (b) growing the transformed host cell under conditions that are suitable for expression of the chimeric gene wherein expression of the chimeric gene results in production of the auxin transport protein encoded by the operably linked nucleic acid fragment in the transformed host cell;
  - (c) optionally purifying the auxin transport polypeptide expressed by the transformed host cell;
  - (d) treating the auxin transport polypeptide with a compound to be tested; and
  - (e) comparing the activity of the auxin transport polypeptide that has been treated with the test compound to the activity of an untreated auxin transport polypeptide,
- 35 thereby selecting compounds with potential for inhibitory activity.
  - 17. A composition comprising the isolated polynucleotide of Claim 1.
  - 18. A composition comprising the isolated polypeptide of Claim 10.

WO 00/68389 PCT/US00/12061

19. The isolated polynucleotide of Claim 1 comprising a nucleotide sequence having at least one of 30 contiguous nucleotides.

- 20. A method for positive selection of a transformed cell comprising:
  - (a) transforming a host cell with the chimeric gene of Claim 5; and
  - (b) growing the transformed host cell under conditions which allow expression of a polynucleotide in an amount sufficient to complement a null mutant to provide a positive selection means.
- 21. The method of Claim 20 wherein the host cell is a plant.
- 22. The method of Claim 21 wherein the plant cell is a monocot.
- 10 23. The method of Claim 21 wherein the plant cell is a dicot.

5

15

- 24. A method of modulating expression of a polypeptide for modulating root development in a plant, comprising the steps of:
  - (a) stably transforming a plant cell with an auxin transport protein polynucleotide operably linked to a promoter, wherein the polynucleotide is in sense or antisense orientation; and
  - (b) growing the plant cell under plant growing conditions to produce a regenerated plant capable of expressing the polynucleotide for a time sufficient to modulate root development in the plant.